Rec'd PCT/PTO 12 MAY 2005

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



10/5345**35**

(43) International Publication Date 3 June 2004 (03.06.2004)

PCT

(10) International Publication Number WO 2004/046184 A1

(51) International Patent Classification⁷:

C07K 14/715,

(21) International Application Number:

PCT/EP2003/050824

(22) International Filing Date:

13 November 2003 (13.11.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 02025755.6

15 November 2002 (15.11.2002) E

(71) Applicant (for all designated States except US): ARES TRADING S.A. [CH/CH]; Le Château, CH-2028 Vaumarcus (CH).

- (72) Inventor; and
- (75) Inventor/Applicant (for US only): ROSSI, Mara [IT/IT]; Via Giuseppe Mantellini, I-00179 Rome (IT).
- (74) Agent: SERONO INTERNATIONAL S.A. INTEL-LECTUAL PROPERTY; 12, Chemin des Aulx, CH-1228 Plan-les-Ouates (CH).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,

[Continued on next page]

(54) Title: PROCESS FOR THE PURIFICATION OF TNF-BINDING PROTEINS USING IMAC

Capture step	CU**-CHELATE	Loading: pH6.8
		Elution: pH 3.0
	Ų.	
Intermediate	SP-SEPHAROSE FF	Loading: pH 3.0
Steps		Elution: pH 4.0
	1	
	10 KD ULTRAFILTRATION	
	Į.	•
	Q-SEPHAROSE FF	Loading: pH 9.0
		Elution: pH 9.0
	1	•
	NANOFILTRATION FOR VIRUS	
	REMOVAL	
	ı,	
Polishing	BUTYL SEPHAROSE FF	Loading: pH 7.5
steps		Elution: pH 7.5
	Ţ.	•
	10 KD ULTRAFILTRATION	
	II.	•
	0.22 μ microfilration	
	Ţ.	•
	r-hTBP-1 BULK	40 mM PBS pH
		70 10 mM NaCI

(57) Abstract: A new purification process for Tumor Necrosis Factor-binding proteins is described. In particular this process is characterized by the use as capture step of an Immobilized Metal Affinity Chromatography (IMAC) using copper as metal. This brings advantages in terms of process yields, purity of the final product and applicability to industrial scale.